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Engineer Research and Development Center

Acute Inhalation Toxicity of Fog Oil Smoke in the Red-winged Blackbird, a Size-specific Inhalation Surrogate for the Red-cockaded Woodpecker

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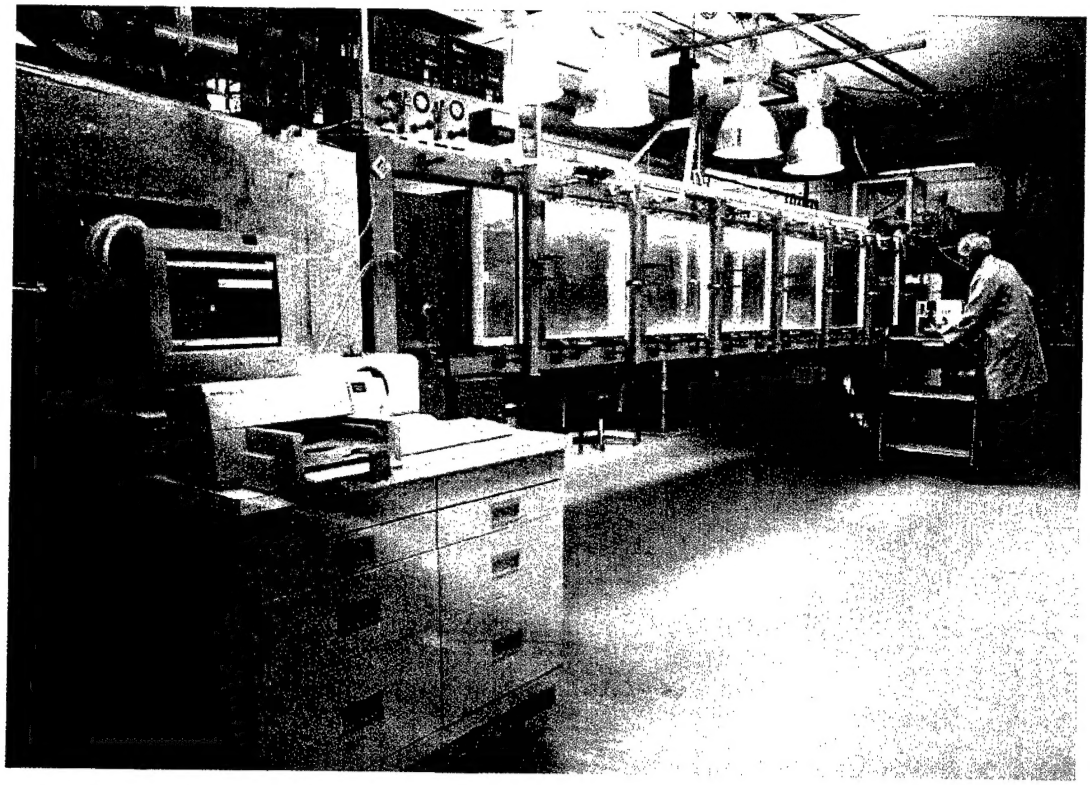

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Cover photo:

Environmental Simulation Wind Tunnel at PNNL Aerosol Research Facility
Pacific Northwest National Laboratory, Richland, WA

Foreword

This study was conducted for the Strategic Environmental Research and Development Program (SERDP) under project number CS-507 "Threatened, Endangered, and Sensitive Resources: Impact of Smokes and Obscurants on TES." The technical monitor for the project was Dr. Femi A. Ayorinde, SERDP Cleanup and Conservation Program Manager, followed by Dr. Robert W. Holst, Compliance and Conservation Program Manager. The executive Director of SERDP is Mr. Bradley P. Smith.

The work was performed by the Pacific Northwest National Laboratory (PNNL), Richland, Washington, under Military Interdepartmental Purchase Requests (W52EU261647000, W52EU28254173, and W2V5AA51305044) from the Ecological Processes Branch (CN-N) of the Installations Division (CN), Construction Engineering Research Laboratory (CERL). The CERL Principal Investigator and contract monitor was Dr. Keturah Reinbold. Patricia M. Kirby, Colorado State University contractor, coordinated preparation of the final report. Dr. Crystal Driver was the PNNL Principal Investigator. Michael Ligotke, Heather Galloway-Gorby, and Gary Dennis also are employed by PNNL. The technical editor was Gloria J. Wienke, Information Technology Laboratory. Dr. Harold Balbach is the Threatened and Endangered Species Research Project Leader. Steve Hodapp is Chief, CEERD-CN-N, and Dr. John T. Bandy is Chief, CEERD-CN. The associated Technical Director was Dr. William D. Severinghaus, CEERD-CV-T. The Director of CERL is Dr. Alan W. Moore.

CERL is an element of the U.S. Army Engineer Research and Development Center (ERDC), U.S. Army Corps of Engineers. The Commander and Executive Director of ERDC is COL John Morris III, EN and the Director of ERDC is Dr. James R. Houston.

Executive Summary

The red-cockaded woodpecker (*Picoides borealis*) is an endangered species that often is found on military lands where troop readiness training is conducted. An important component of troop readiness training is the generation of obscurant materiel and conduct of maneuvers under obscurant cover. Fog oil (SGF-2) is the obscurant most commonly deployed for these purposes. To aid in assessing and managing the impact of fog oil to sensitive wildlife species, such as the red-cockaded woodpecker, we evaluated the acute toxicity and health risk of environmental releases of fog oil to avian wildlife. The red-winged blackbird (*Agelaius phoeniceus*) was used as a surrogate species for the red-cockaded woodpecker because of its sensitivity to chemicals, its history of use by U.S. Fish and Wildlife Service and U.S. Environmental Protection Agency as a representative wildlife species in risk evaluation of environmental contaminants, and its similarity in physical size to the red-cockaded woodpecker. This similarity in body size provides for comparable minute ventilation, feather mass, and body surface area, characteristics that largely determine the respiratory, preening, and dermal uptake of airborne contaminants by birds.

Wild red-winged blackbirds were exposed to airborne concentrations of fog oil ranging from typical field concentrations (50 and 100 mg/m^3) to levels present only within the proximity of a stationary generator (400 mg/m^3). Control birds were exposed under the same conditions but without fog oil in the air stream. The post-exposure response of blackbirds was monitored for 28 days. Body weights were obtained prior to exposure, at exposure, and at 5 days and 28 days post-exposure. Half of the birds were sampled for gross necropsy and tissue pathology at 1 week post-exposure and half at 4 weeks post-exposure. Respiratory function was measured in a subset of birds prior to exposure and during exposure. Respiratory abnormalities were monitored by observation during the post-exposure period. The birds were also observed twice daily for clinical signs of toxicity, including behavioral changes.

Because the polynuclear aromatic hydrocarbon (PAH) content of fossil fuels may contribute significantly to the toxicity of refined oils and because they can be products of pyrolysis (chemical change brought about by the action of heat), the

researchers determined the PAH content of the both stock fog oil and fog oil aerosols. Compositional changes in the smoke, compared to the stock oil, were found and consisted primarily of a 97 percent decrease in naphthalene concentration and a slight increase in the number and concentration of high molecular weight PAHs. Although naphthalene is a respiratory irritant in mammals, in birds it is highly toxic when inhaled, therefore the potential toxicity of the fog oil, relative to naphthalene exposure, was diminished for birds by the generation process. Airborne concentrations of high molecular weight PAHs in the 400 mg/m^3 fog aerosol were calculated and found to be very low (≤ 0.02 mg/m^3). For more field-typical exposures (100 mg/m^3 of fog oil), the airborne concentration of PAH is estimated to be about 0.005 mg/m^3.

To estimate oral exposure from preening, fog oil and PAH deposition to feathers of taxidermic birds were determined. Fog oil (and PAH) deposition on feathers was measurable but small, indicating that oral uptake via preening of contaminated feathers is a minor route of exposure for birds. No detectable levels of the more toxic high molecular weight PAHs were found on the feathers of taxidermic birds.

Compared to control birds, no mortality, body weight loss, clinical signs of toxicity, or behavioral abnormalities were observed in any of the fog oil-treated birds. Gross and histopathological lesions were observed in the red-winged blackbirds; however, the distribution and severity of all lesions were independent of the fog oil exposures and were typical of those found in wild bird populations. Although respiratory function data were limited, no major respiratory impairment or abnormalities were observed during the exposure or the post-exposure period.

Data from this study indicate that (1) the acute toxicity of inhaled fog oil from obscurant aerosols is low for adult red-winged blackbirds, and (2) uptake of SGF-2 is minimal for blackbirds and birds of similar size-specific inhalation rates, feather mass, and dermal surface areas. No observable adverse effects were seen in birds exposed to fog oil concentrations up to about 400 mg/m^3, a worst-case exposure scenario of birds remaining in close proximity to a generating system for extended periods (up to 4 hours).

Contents

List of Figures and Tables

Figures

Tables

1 Introduction

Background

Domestic Army installations, when combined with installations of other U.S. military services, total more than 25 million acres (about 10 million hectares). Among this land area are significant parcels where the intensity of use is low enough, or infrequent enough, to allow development of populations of species considered rare outside the installation. When the aggregate area of essential or preferred habitat is adequate to support their population, threatened and endangered wildlife species often find refuge on military lands. Therefore, essential military functions occurring on military bases must coincide with efforts ensuring the continued health of the critical populations of these species, while maintaining operational constraints of the installations. One such challenge falls under the Endangered Species Act of 1973 (PL 93-205; 16 USC 1531 et seq., as amended), which promotes the maintenance/expansion of red-cockaded woodpecker (*Picoides borealis*) populations in areas of troop readiness training.

Among the training activities that occur in or near woodpecker habitat is the generation of aerosols of obscurant materiel. Obscurants have long been used to mask movements of troops and mechanized equipment. Of the conventional smokes, the white smoke generated from vaporization and condensation of liquid fog oil (SGF-2) is an effective obscurant in the visible range. It is the most heavily used obscurant for troop training because of its low cost, ease of handling and smoke generation, dispersion characteristics, and safety (Eberhard, Cupp, and Abshire 1989).

It must be noted that the term "fog oil smoke" is somewhat misleading. While the white clouds emitted from the generators are commonly called "smoke," they really are better described as "fog," and are composed of small droplets, not unlike a fog composed of water droplets. Ideally, no combustion products are present in this fog, and the oil is not "burned" by any process. In practice, however, the heated portions of the generator may cause thermal recombination in a small portion of the fog oil, and the engine powering the military generating equipment may contribute some exhaust products.

Troop preparedness training and the field testing of generating equipment often involves release of fog oil into the atmosphere. Managing sensitive avian populations, in conjunction with providing realistic troop training opportunities, requires an accurate assessment of the toxicity and health effects of fog oil to species such as the red-cockaded woodpecker. However, the effects of airborne fog oil on wildlife and, in particular, avian wildlife are poorly known or unknown. It is important to determine potential adverse effects in birds because they are often more sensitive to airborne pollutants than mammals, have high public visibility, and are used as bio-indicators of ecosystem health.

Prior to the conduct of the studies reported here, the toxic effects of airborne fog oil aerosols on wild birds could only be inferred from studies of aquatic (predominately marine) oil-spills and from laboratory studies simulating oral and dermal exposures from such spills. These data are often conflicting due to significant differences in petroleum composition, dosing regime, bird species, and age class tested (Clark 1984). Virtually no data are available on the inhalation toxicity of petroleum-based oils or polynuclear aromatic hydrocarbons (PAHs, the more toxic components of petroleum oils) in birds. Inhalation of fog oil aerosols has been investigated in human surrogate species (for example, laboratory rodents) and harmful exposure concentrations extrapolated for humans. However, the application of these values to the wide variety of wild animal species is problematic because both the sensitivity to air pollutants and the doses received (on a body-weight basis) differ greatly among species (Hill 1994; Schafer 1972; Schafer, Bowles, and Hurlbut 1983). For example, the volume of air breathed per minute per unit of body weight (the weight-specific minute ventilation) varies greatly among mammals (Phalen 1984). Generally, the smaller the animal, the more air per minute per gram is inhaled. Compared to humans, rabbits ventilate 3 times the volume of air and small rodents ventilate 8 to 13 times the volume, on a per-body-weight basis (Phalen 1984). Birds may be at greater risk of exposure because their respiratory rates are generally higher than those of mammals of comparable size. However, because of fundamental differences in their respiratory systems, mammalian inhalation data cannot be extrapolated to avian species. Similarly, feather deposition and subsequent oral uptake via preening cannot be estimated from mammalian studies. For bird species with critical populations (threatened and endangered species), exposure and toxicity are best estimated from size-specific surrogates.

From the available oil-spill studies, the susceptibility of birds to the toxic effects of petroleum from oral and dermal exposure appears to be much greater than that of many other organisms (Geraci and Smith 1977; Grau et al. 1977; Hartung and Hunt 1966; Hartung 1967; Chia 1971; and Rowe, Dollahite, and Camp 1973)

and refined oils are more toxic to birds than are crude oils (Fleming, Sileo, and Franson 1982). This inordinate susceptibility probably results from the ability of feathers to readily absorb large quantities of oil immediately upon contact. Absorption of the oil by the feathers retains oil for ingestion through preening (and contamination of eggs in nesting birds) and deprives the birds of the critical functions of their feathers (such as insulation, water repellency, and flight). The impact of oil aerosols, such as SGF-2 obscurant, on feather function and subsequent health deficits has not been investigated. However, given the absorption capability and sensitivity of feathers to oil contamination, such studies are necessary for accurate hazard assessments.

Objectives

The objectives of this study were to evaluate the health effects of fog oil aerosols in a surrogate species for the red-cockaded woodpecker, and to provide information for general and site-specific risk assessments, and the management of obscurant fog oil generations and training activities. Because airborne fog oil can also deposit on feathers and subsequent preening of oiled feathers can result in dermal and indirect oral uptake of fog oil in addition to the inhaled dose, birds received both respiratory and feather/dermal exposures.

Scope

The studies described in this report are limited to examination and discussion of acute inhalation toxicity of adult surrogate birds of both sexes. No individuals of threatened or endangered species were used in this study. Additional studies have been performed to examine the effect of fog oil smoke on hatchlings and nestlings of other surrogate avian species, as well as examination of the survivability of the fledglings as adults, and of their reproductive success, and examination of possible abnormalities in successive generations. Reports covering these additional studies are in preparation, and these aspects of the relationship between avian species and the military use of fog oil smoke will be published at a later date.

Approach

The avian species of direct concern is, as stated earlier, the red-cockaded woodpecker (*Picoides borealis).* Since this is a listed species, and because no other roughly similar woodpecker species was available in populations adequate to

conduct the proposed studies, a species surrogate was selected for this study. The red-winged blackbird (*Agelaius phoeniceus*) was selected because of its general acceptance in the scientific and regulatory community as an adequately-sensitive wild bird species for toxicity tests involving environmental contaminants (Schafer 1972; Schafer et al. 1983; Schafer 1994).

In addition to its sensitivity to a wide range of compounds, the red-winged blackbird was selected as the test species because it has a body length and body weight similar to the red-cockaded woodpecker. The similarity in body size should result in similar weight-specific minute volumes and, consequently, similarity in inhaled dose (Phalen 1984). Because feather mass and body surface area can be expressed as a function of body mass in birds (Calder and King 1974; Turcek 1966; Walsberg and King 1978), contamination of these surfaces/exposure routes should also be similar between the two species.

Wild, adult red-winged blackbirds were baited to traps and transferred to an outdoor aviary at Pacific Northwest National Laboratory (PNNL) 6 weeks prior to testing. Birds were fed a pelleted, complete diet for insect-eating soft-billed birds, with enrichment from fresh plant materials from time to time. The aviary perches, roost boxes, water troughs, and floor were disinfected daily. Natural perches were hosed-down with water.

Male and female blackbirds were assigned to pens using a random numbers table (Zar 1974). Body weights were measured 2 weeks prior to testing. Statistical tests showed that all the flight pen population variances for each sex were homogeneous; no significant differences existed for the mean body weights of all test groups ($P \geq 0.05$) for each sex.

Tests were conducted at the PNNL Aerosol Research Facility. Three concentrations of fog oil were used in the inhalation tests and included typical-to-high field generation concentrations (target concentrations of 50, 100, and 400 mg/m^3). Birds were exposed for either 2 or 4 hours. During a 4-week post-exposure period, observations were made for mortality, body weight change, behavioral abnormalities, and potential signs of toxicity associated with the aerosol exposure. Birds were examined for gross lesions and histopathological damage at 1 week and 4 weeks after exposure.

An estimate of the amount and composition of fog oil available for ingestion through feather preening was made by placing taxidermic red-winged blackbirds in the control and test chambers during a 1-hour generation. Fog oil concentrations per gram of feathers were reported and the quantity deposited on the bird

estimated. In an additional baseline study, and because the PAH content of fossil fuels may contribute significantly to the toxicity of refined oils and because they can be products of pyrolysis, the PAH content of the stock fog oil and fog oil aerosols used in the exposure studies was determined.

The responses of the birds to the oil aerosol exposures were monitored by measuring mortality rate, nineteen separate clinical signs of toxicity, body and liver weight changes, behavioral and respiratory function changes, and the gross and histopathological examination of organs and tissues. Respiratory physiology was monitored in 12 additional birds per group (half males, half females). Visual signs of abnormal respiratory function (excessive panting, deep chest movements, gaping) were monitored during the observation periods.

Behavioral observations were made twice daily for 1 week prior to fog oil exposure and for Days 0 through 5 and Days 22 through 28 after exposure. In addition to general signs of stress or disease, the observers were instructed to look for behavior that has been observed in birds dosed with crude or refined oils (Hartung 1967; Croxall 1977; Fleming, Sileo, and Franson 1982).

Mode of Technology Transfer

The information included in this report is one portion of the materials prepared by the Engineer Research and Development Center (ERDC) to assist installation natural resource and Threatened and Endangered Species program managers. The primary means of communicating the inhalation toxicity information will be through publication in the scientific literature, as well as through the availability of this report. The specific data presented are intended to be used in the preparation of biological opinions related to planned Army actions where the red-cockaded woodpecker (or other similar avian species) is present, and for endangered species management plans (ESMPs), integrated natural resource management plans (INRMPs), and in the preparation of ecological risk assessments involving fog oil smoke and avian species.

This report will be made accessible through the World Wide Web (WWW) at URL: http://www.cecer.army.mil

2 Methods

Test Animals

The red-winged blackbird (*Agelaius phoeniceus*) was selected for this study because of its general acceptance in the scientific and regulatory community as a sensitive wild bird species for toxicity tests involving environmental contaminants (Schafer 1972, Schafer 1994). For example, in a study of the acute toxicity of 998 compounds to 68 species of wild and domestic birds, the red-winged blackbird was shown to be the most sensitive of the species tested (Schafer, Bowles, and Hurlbut 1983).

In addition to its sensitivity to a wide range of compounds, the red-winged blackbird was selected as the test species because it is similar in physical size to the red-cockaded woodpecker. Male red-winged blackbirds range in length from 20 cm to 25 cm (7.0 in. to 9.5 in.) with a continental average body length of about 23 cm (9 in.). Females are about 20 percent smaller than the males (Beletsky 1996). The average body length of the red-cockaded woodpecker has been reported as about 22 cm (8.5 in.). Body length and wing length are considered good indicators of size because they do not change seasonally, but are highly correlated with body mass (Connell, Odum, and Kale 1960; Searcy 1979). Depending on the month during breeding season, nutritional status, and geographical location, the body mass of male red-winged blackbirds ranges from 60 g (1.93 oz) to 88 g (2.83 oz) (Beletsky 1996). Male red-cockaded woodpeckers have an average body mass of 57 g (1.83 oz) (Jackson 1971). The similarity in body size should result in similar weight-specific minute volumes and, consequently, similarity in inhaled dose (Phalen 1984). Because feather mass and body surface area can be expressed as a function of body mass in birds (Calder and King 1974; Turcek 1966; Walsberg and King 1978), contamination of these surfaces/exposure routes should also be similar between the two species.

Source and Husbandry of Test Animals

One hundred forty wild adult red-winged blackbirds were baited to traps in Benton and Franklin counties, Washington, and transferred to an outdoor aviary at Pacific Northwest National Laboratory 6 weeks prior to testing. The birds were

housed in a 9.1-m wide by 15.2-m long by 3.7-m high (30-ft by 50-ft by 12-ft) aviary that was divided into 5 flight pens, each 3 m (10 ft) wide. A metal roof covered one-third of each section. Continuously flowing water was provided for drinking and bathing/swimming in each flight pen by capping the end of a 3-m (10-ft) long PVC gutter and providing a constant flow of water into one end of the gutter. Spillover ran across the width of each pen to a drain and provided a wading area. Wooden rods suspended from the roof frame provided perches for the birds in covered portions of the aviary. Natural and artificial evening roosts in the covered area consisted of fir (*Abies* spp.) and spruce (*Picea* spp.) trees placed in planters and arranged around 4-foot-high roost boxes. Crabapple trees (*Malus* spp.), spruce trees, and rhododendrons (*Rhododendron* spp.) provided natural cover in the open areas of the flight pens.

Birds were fed a pelleted, complete diet for insect-eating soft-billed birds (Zeigler Bros, Inc., Gardners, PA, Product No. 73534800). Minimum values for crude protein and crude fat content of the semi-moist feed were 30.0 percent and 12.0 percent, respectively. Maximum crude fiber was 4.0 percent, maximum moisture was 15.0 percent, and the maximum ash content was 8.0 percent. Although the diet was complete, fresh corn (on the cob), unbleached rice, grapes, natural millet sprays, and lettuce seedlings were also provided for dietary variety and environmental enrichment. The aviary perches, roost boxes, water troughs, and floor were disinfected daily. Natural perches were hosed down with water.

A laboratory animal veterinarian observed the blackbirds during the quarantine and 6-week acclimation period, found them in good health, and released them for study at the end of the acclimation period.

Bird Identification and Group Assignment

For 7 days after capture, all 140 blackbirds were housed in 1 flight pen. On the 8th day, 16 females and 16 males were transferred to each of the remaining 4 flight pens. Surplus birds were housed in the 5th pen during the subsequent 6-week acclimation period, to serve as aviary controls and as a pool for replacing any birds injured in the test pens (no replacements were made 2 weeks prior to testing). To uniquely identify each bird, a 2-mm x 12-mm barcode transponder (AVID Company, Norco, CA), was implanted into the pectoral muscle of each bird using a needle injector. The transponders were stored in 70 percent ethanol until implanted. A povidone-iodine solution applied to the skin at the implant site acted as an antiseptic prior to injection of the transponder. When necessary, individual birds were identified using a barcode reader (AVID Company, Norco, CA).

Male and female blackbirds were assigned to pens using a random numbers table (Zar 1974). Body weights were measured 2 weeks prior to testing. The data were analyzed for homogeneity of variance using Bartlett's test, and for differences in mean body weights among test groups using one-way analysis of variance (ANOVA, Zar 1974). The results of these statistical tests showed that all the flight pen population variances for each sex were homogeneous; no significant differences existed for the mean body weights of all test groups ($P \geq 0.05$) for each sex. Therefore, the group assignments were retained for the study.

Fog Oil Exposures

At the beginning of the study, birds were transferred to the PNNL Aerosol Research Facility at 0945 hrs and exposed to control or fog oil aerosols within 30 minutes of arrival. Three concentrations of fog oil were used in the inhalation tests and included typical-to-high field generation concentrations (target concentrations of 50, 100, and 400 mg/m^3), based on predictions from a modified Gaussian plume dispersion model (Driver et al. 1993). Birds were exposed for either 2 or 4 hours. Test conditions, aerosol characteristics, and bird behaviors were monitored during the exposure tests. After exposure, the birds were returned to their assigned flight pens in the outdoor aviary. During a 4-week post-exposure period, observations were made for mortality, body weight change, behavioral abnormalities, and potential signs of toxicity associated with the aerosol exposure. Birds were examined for gross lesions and histopathological damage at 1 week and 4 weeks after exposure.

Fog Oil

A variety of fog oils have been used to provide white smoke obscurant; however, SGF-2 is the fog oil that has been in use for year-round obscuration needs for the past 20 years (U.S. Army 1986). In 1986, military specification MIL-F-12070C specified procurement and use of fog oil with reduced quantities of some of the potentially harmful components of the materiel (for example, PAH). SGF-2 used in this study was Lot number 71808 manufactured by American Lubricating Company, Memphis, TN in March 1991 and supplied to PNNL by U.S. Army Edgewood Research, Development, and Engineering Center (ERDEC, now a part of the U.S. Army Soldier and Biological Chemical Command [SBCCOM]), Aberdeen Proving Ground, MD. The oil had a test date designation of March 1994. Upon receipt, the oil was assigned a unique and monitored barcode through the PNNL Chemical Management System. Stock and aerosolized fog oil was analyzed by gas chromatography/mass spectrometry (GC/MS) as described in the following section.

Analysis of Fog Oil for Polynuclear Aromatic Hydrocarbons

Because the toxicity of petroleum oils is often related to the quantity of PAH present, samples of fog oil were collected, prior to generation, for chemical characterization of the stock oil. Samples were also taken at entry into the test chambers during generation of fog oil smoke to determine if the smoke generation process (high heat vaporization) produced an aerosol of different composition than that of the stock oil. Aerosol samples were collected for 2 or 3 hours during preliminary generations of the 400 mg/m^3 fog oil test concentration. Samples were collected on aluminum foil deposition coupons (929 cm^2). Unused foil coupons and foil coupons placed in control test chambers that received output from the generation system described in the section on fog oil generation (see page 18), but without fog oil, were used as blanks. All samples were placed in bottles with Teflon™-lined lids and stored at -20° C (-4 °F) until analyzed for PAH content. Glassware and foil were heated to 400° C (752 °F) for 24 hours; Teflon™-lined lids were rinsed with GC grade hexane and methylene chloride prior to use.

Fog oil samples were extracted with methylene chloride according the National Oceanic and Atmospheric Administration Status and Trends Program Technical Memorandum NMFS F/NWC-153 (Krahn et al. 1988). Samples were then cleaned using silica/alumina (5 percent deactivated) chromatography, followed by High Pressure Liquid Chromatography (HPLC) cleanup. Selected deuterated surrogate PAH compounds were added at the beginning of each extraction to assess the efficiency of the method; all results were corrected for the recoveries of the deuterated surrogates. Extracts were quantified using GC/MS in the selected ion mode (SIM), following a procedure based on U.S. Environmental Protection Agency (USEPA) method 8270 (USEPA 1986).

Exposure Chambers

Four Plexiglas™ chambers were constructed within the PNNL Aerosol Research Wind Tunnel (see cover photo). Each chamber was cubical (61 cm [24 in.] on all sides) with a volume of 0.23 m^3 (8.12 cu ft). One exhaust and two inlet lines were attached to the upper portions of each chamber. Two additional ports were installed in the chambers to obtain physical samples. The ports also allowed a small flow to be withdrawn and passed to optical dust sensors for real-time monitoring of aerosol concentrations. The ambient temperature and humidity of the exposure chambers were maintained at values similar to those at the aviary during the same period of the day. The target values were obtained by calculating the mean temperature and humidity recorded at the aviary between 0900

and 1100 hours for the 5 days just prior to the day of exposure. Temperature and humidity data were collected. The mean temperature and relative humidity during exposure were 18.0° C (17.5° C to 18.1° C) and 42.3 percent RH (41.7 RH to 44 percent RH), respectively.

Decreased light penetration, achieved by covering the external walls of the chambers, reduced stress and limited startle responses in the birds during testing. One wall of the chamber was movable and was outfitted with removable perches. To move the birds with minimum stress out of the chamber and into transfer cages, the perches were pulled out of the chamber side of the wall and affixed to the exterior side of the moveable wall. The perches were used as handles to slowly move the wall, herding the birds toward the opposing wall that contained the transfer portal and into a transfer cage. Transfer cages were covered with a shroud. The birds were returned to their home aviary within 15 minutes of transfer.

Fog Oil Generation

Fog oil aerosols were generated by metering steady rates of liquid fog oil onto a heated immersion element maintained at 600° C (1112 °F) and contained within a 1-m-long, 2.5 cm-diameter stainless steel pipe (Figure 1). Vaporized oil was carried in a mixture of nitrogen (96 percent) and air (4 percent) through a region controlled at 300° C (556 °F) into a buffer tank with a residence time of 5 minutes. In the buffer volume, fresh air was mixed with the concentrated fog oil aerosol and the mixture drawn into the test chambers in the wind tunnel at ambient temperature (18° C, 64 °F). Valves were used to adjust the flow of aerosol into each of three exposure chambers. To ensure mixing, restrictions were installed at the aerosol inlet to each chamber. The restrictions caused the fog oil aerosol to jet into the upper regions of the chambers and then quickly mix to a uniform concentration at the breathing zone height of the birds. The feed rate of the oil was adjusted periodically, based on sensor-monitored aerosol concentration (see the section titled "Fog Oil Concentration and Droplet Size" on the following page), to maintain the test concentrations.

Exposure Characterization

In addition to the chemical characterization of the generated SGF-2 (see "Analysis of Fog Oil for Polynuclear Aromatic Hydrocarbons," page 17), the fog oil concentration and the droplet size distribution of the aerosols were characterized during the test exposures. To characterize the preening/dermal exposure of the birds, the deposition rate of oil to feathers and the chemical composition of the deposited material were also determined.

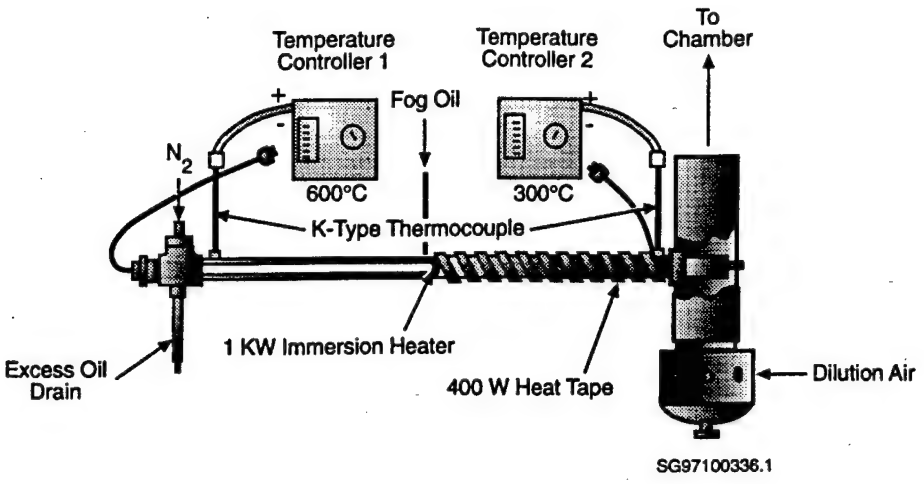

Figure 1. Temperature-controlled fog oil aerosol generator.

Fog Oil Concentration and Droplet Size. Pre-test generations of fog oil were conducted under the same conditions as the test generations to determine the relative mass of the vapor and droplet components. Occupational Safety and Health Administration (OSHA) Versatile Samplers (OVS; SKC West, Inc., Fullerton, CA) were used for simultaneous collection of aerosol and vapor. In this collection system, glass fiber filters collect the aerosol; a two-section sorbent bed of XAD-2 is used to absorb vapor. The 400 mg/m^3 exposure chamber was sampled for 10 minutes at a flow rate of 250 milliliters per minute (mL/min). Samples were weighed to the nearest 0.01 mg on a Mettler Model AE163 analytical balance. The mass of sample collected on the OVS tubes was compared to the mass collected on glass filter fibers without the sorbent. The mass difference was the mass of the vapor component in the airborne fog oil. Results showed the mass of the vapor was below detection (≤0.05 mg). Therefore, particle-count and aerosol mass methods were used during the test generations to quantify exposure.

The concentration of fog oil aerosol in each of the test chambers was monitored in real time using M.I.E. Model IDS-10 Optical Dust Sensors (Monitoring Instruments for the Environment, Inc., Billerica, MD). Simultaneous gravimetric samples were taken by drawing chamber air through pre-weighed, 47-mm high-efficiency glass fiber filters (Gelman, Ann Arbor, MI) at 1 Liter per minute (Lpm) for 15 minutes. The filters were weighed to the nearest 0.1 mg on a Mettler Model AE163 analytical balance prior to and after sample collection to determine the mass collected. Airborne fog oil concentrations were reported in mg/m^3. The mean of the optical dust sensor values collected during the gravimetric sampling period was compared to the gravimetrically derived air concentrations. Least

squares linear regression was used to test the relationship between the optical dust sensor millivolt (mV) readings and the gravimetric air concentrations (Figure 2). The relationship was linear ($r^2 = 0.98$) and the equation was used to convert the sensor readings into the air concentration values during the exposures. Aerosol concentrations were also measured gravimetrically at 1-hour intervals during the exposures as a crosscheck on the sensor readings.

To assure droplet sizes comparable to those generated in the field, the particle size distribution of the fog oil aerosols was measured for the 100 mg/m³ and 400 mg/m³ aerosol concentrations using Andersen cascade impactors operated at a flow rate of 28 Lpm. All aerosol samples were drawn from the chamber center at the height of the birds' breathing zone.

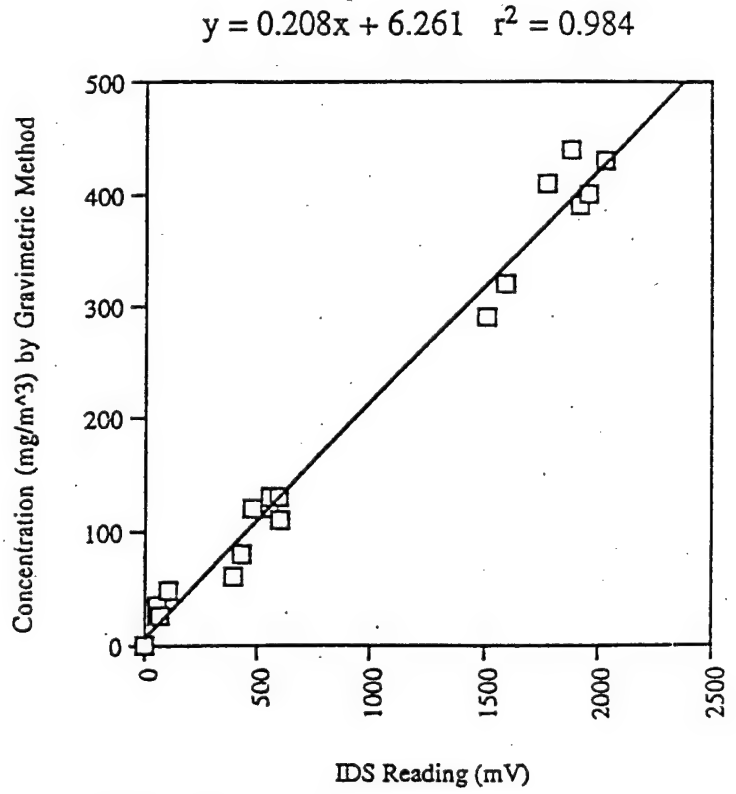

Figure 2. Relationship between millivolt readings of the optical dust sensor and the mass of fog oil collected on glass fiber filters.

Fog Oil Composition and Deposition on Feathers. An estimate of the amount and composition of fog oil available for ingestion through feather preening was made by placing taxidermic red-winged blackbird specimens in the control and test chambers during a 1-hour generation. The feathers of the bird specimens were then removed and placed in solvent-rinsed jars with Teflon™-lined lids and stored at -20° C (-4 °F) until analysis. Fog oil concentrations per gram of

feathers were reported and the quantity deposited on the bird estimated from the following equation:

$$Y=0.09M^{0.95} \quad \text{[Equation 1]}$$

Where:

Y = the plumage weight in grams

M = the average body weight in grams (Turcek 1966).

Ions 43 and 57, which are the predominant ions in alkanes (principle component of fog oil), were used to identify the fog oil on the feathers by GC/MS, as described in the section on analysis of fog oil (see page 17). Ion 57 was used as the quantifying ion. However, some late eluting compounds (probably natural feather oils) with ions 43 and 57 were found in the feather extracts. Summing only the area under the peaks that eluted between 20 and 40 minutes circumvented potential exaggeration of the fog oil value from this matrix. Integration of this area appeared to account for all the fog oil in the feathers, while excluding the natural oils.

Response Measures

The responses of the birds to the oil aerosol exposures were monitored by measuring mortality rate, clinical signs of toxicity, body and liver weight changes, behavioral and respiratory function changes, and the gross and histopathological examination of organs and tissues.

Mortality and Clinical Signs of Toxicity

Observations for clinical signs of toxicity, mortality, and moribund birds were recorded twice daily (before 1000 hrs and after 1500 hrs) during normal work days and at least once daily on weekends. In addition to overall condition, observers were required to check for, but were not limited to, the following clinical signs:

- hyperactivity/hypoactivity
- emaciation
- abnormal posture
- nasal discharge
- dyspnea
- alopecia
- swelling
- hypothermia
- dehydration
- tremor
- gular flutter
- excessive preening
- cloacal stain
- feather loss
- fecal discoloration
- eye irritation or sunken appearance
- fluffed feathers
- excessive face and beak rubbing
- ataxia

Pathology

Half of the males and half of the females of the control and of each test group were sacrificed 1 week after exposure and the remaining birds at 4 weeks after exposure. A gross necropsy was performed on each test bird. The blackbirds were sacrificed by carbon dioxide asphyxiation and immediately the ceca and intestine were injected with 10 percent buffered formalin to prevent autolysis. All major organs were examined for gross lesions. The breast muscle was examined for atrophy and the terminal body weight, general body condition, and presence of body fat recorded. Air sacs were viewed for lesions and clarity of tissue. The skin, lung, trachea, thyroid, thymus, heart, liver, spleen, proventriculus, pancreas, small and large intestine, gizzard, cecum, kidney, and gonad were excised and preserved in 10 percent neutral buffered formalin. The gender of the bird and its reproductive status were confirmed during necropsy. Tissues from eight birds (4 females and 4 males) from each test (2-hour exposure and 4-hour exposure) were prepared for histological examination and were read by an avian specialist at the Veterinary Diagnostic Laboratory at the College of Veterinary Medicine, Oregon State University. Histologic slides were read without specific knowledge of the treatment of individual birds.

Body Weight and Liver Mass

Body weights were measured to the nearest gram on a PESOLA metric spring scale (100 g [3.22 oz] or 300 g [9.65 oz] capacity) at 2 weeks prior to exposure, at exposure, and at days 5 and 28 after treatment. Liver weights were obtained for birds in the 4-hour exposure study. The tissue mass was determined to the nearest milligram at necropsy using a Mettler AE260 analytical balance. Prior to each weighing session, the scale and balance calibrations were checked using a calibrated Troemner (Philadelphia, PA) metric weight set (2 mg to 100 g). The balance and weight set calibrations are traceable to National Institute of Standards and Technology standards.

Body weight data were analyzed using one-way ANOVA to test for differences in mean body weights among test groups for each sex. Liver weight was expressed on a percentage-of-body-weight basis and an arcsine transformation applied to this proportion. The arcsine-transformed data were also tested by ANOVA. Treatment group means were compared to the mean weight of the control group for each sex by Dunnett's test (Dunnett 1955; Zar 1974).

Respiratory Function

Respiratory physiology was monitored in 12 additional birds per group (half males, half females). These birds were placed in holding-tubes/plethysmographs inset into the exposure chamber walls, so the heads of the birds protruded into the exposure chamber. The holding-tube was a plastic bottle of about 400 ml volume. A hole was cut into the screw cap to provide an opening for the bird's head. A piece of rubber dental dam with a 6-mm hole punched in the middle and stretched over the bird's head provided a seal around the bird's neck, as well as a seal between the exposure chamber and the bird holding-tube. The holding tube effectively isolated the bird's body surfaces from the head, and acted as a plethysmograph for respiratory function measurements. Respiratory rates of birds restrained in the holding tubes were similar to those of resting birds in their home pens. The birds did become "trap wise," however, and by the second session (Day 0 of exposure) attempted to withdraw into the bottle. The birds became increasingly successful with each measurement session, such that minute ventilation (the volume of air breathed) could not be measured during post-exposure periods. Only observational respiratory rates and abnormalities were collected post-exposure. Because the birds used in the respiratory function tests did not receive a whole-body exposure, their tissues were not evaluated for histopathological lesions and their body weight data was not included in the body mass comparisons shown in the tables in Chapter 3.

A Validyne™ system was used to monitor the respiratory physiology of the blackbirds in the holding-tubes during pre-exposure conditioning and exposure. The system was comprised of the following parts:

- MC1 module rack
- CD18 amplifier set to full span to ensure inspiration/expiration separation
- FV 156 integrator with a span calibrated with a syringe to 4 mL/volt
- DP 103 (0.02 pounds (per) square inch differential [PSID]) transducer that provided a signal strong enough to drive the integrator during the shallow, quiet breathing of the blackbirds
- Fleish 3.0 pneumotachograph connected to the holding-tube by a short piece of 0.25-inch i.d.(interior diameter) tubing.

A Tektronix 5031 oscilloscope was used to measure the Validyne output. An Esterline Angus recorder was used to record respiratory rate, tidal volume (the volume of air inspired or expired with each normal breath), and total volume of the birds.

To test the performance of the plethysmograph, the holding-tube was ventilated with a Harvard model 680 Rodent Respirator set for a resting tidal volume of 0.4 mL/breath. The empty plethysmograph was tested for frequency response over the range of 50 to 200 breaths per minute and consistently registered 0.40 to 0.41 mL/breath. A small taxidermic bird form was placed inside the holding-tube to reduce the volume of the plethysmograph and simulate the space occupied by an adult blackbird. Ventilation was continued without disturbing the ventilator. Tidal volume was 0.40 breaths/min. The test was repeated with the same results, indicating the volume of the bird did not alter the performance of the plethysmograph.

Visual signs of abnormal respiratory function (excessive panting, deep chest movements, gaping) were monitored during the observation periods.

Behavior

Behavioral observations were made twice daily (see page 21) for 1 week prior to fog oil exposure and for Days 0 through 5 and Days 22 through 28 post-exposure. Two independent observers, outfitted with Swarnovski/Habicht SL 10 X 50 binoculars, were stationed at camouflaged viewing stations at opposite sides of the aviary and began observations 20 minutes after entering the station. Birds were observed for 1 hour in the morning and for 1 hour in the late afternoon. In addition to general signs of stress or disease (see page 21), the observers were instructed to look for behavior that has been observed in birds dosed with crude or refined oils (Hartung 1967; Croxall 1977; Fleming, Sileo, and Franson 1982). These behaviors included thermal deficient behavior, such as increased feeding and lethargy, and postural abnormalities where the head is held close to the body and the breast held lower than normal during walking or standing.

3 Results and Discussion

Exposure Characterization

Mean airborne concentrations of SGF-2 and droplet sizes of the exposures were comparable to those measured during, or predicted for, field tests (Driver et al. 1993). The high exposure concentrations were similar to near-source values. PAH composition changed somewhat as a result of the generation process; but appeared to be low for both inhaled and oral (preened) uptake. Estimated fog oil ingestion from preening was also low.

Fog Oil Concentration and Droplet Size

Mean fog oil concentrations during the 2-hour exposures were 54 ±3 mg/m^3, 106 ±23 mg/m^3, and 347 ±27 mg/m^3 from gravimetric analysis. The exposure profiles determined from constant optical sensor monitoring during the 2-hour exposure are shown in the Appendix. Mean airborne concentrations of fog oil determined from the IDS readings were 28 ±5 mg/m^3, 121 ±12 mg/m^3, and 338 ±31 mg/m^3 for the low, mid, and high concentrations, respectively. Continuous concentration values profiled during the 4-hour exposure are also shown in the Appendix. Mean concentrations for the low, medium, and high 4-hour exposures were 34 ±10 mg/m^3, 107 ±27 mg/m^3, and 376 ±56 mg/m^3, respectively.

Aerodynamic mass median diameters of the aerosols were about 1.3 μg, with a geometric standard deviation of 1.5 (Figure 3). These droplet distributions are typical of fog oil aerosols generated in the field (Driver et al. 1993).

Concentration of Polynuclear Aromatic Hydrocarbons in SGF-2 Aerosols

PAH constituents of both the pre- and post-generation fog oil were low in concentration, totaling less than 0.71 ppm of the oil (Table 1). Although the concentrations of most of the constituent PAHs remained the same after the heating process, some compositional changes were observed in the aerosol form of fog oil when compared to ungenerated SGF-2 (Table 1). In general, the amount of high molecular weight PAHs increased as a result of the generation process (Table 1).

Pyrene concentrations increased about six-fold and benzo(a)anthracene and benzo(g,h,i)perylene that were not found in the pre-generation oil were observed in the generated aerosol. Of the low molecular weight PAHs, naphthalene, a compound that sublimes appreciably at temperatures above 80° C (176 °F), was decreased by greater than 97 percent during the generation process. Fluoranthene concentrations increased by two-fold in the aerosolized fog oil; acenaphthylene (not present in pregeneration oil) was found in the aerosol, albeit in low concentrations (1.6 to 6.0 μg/g).

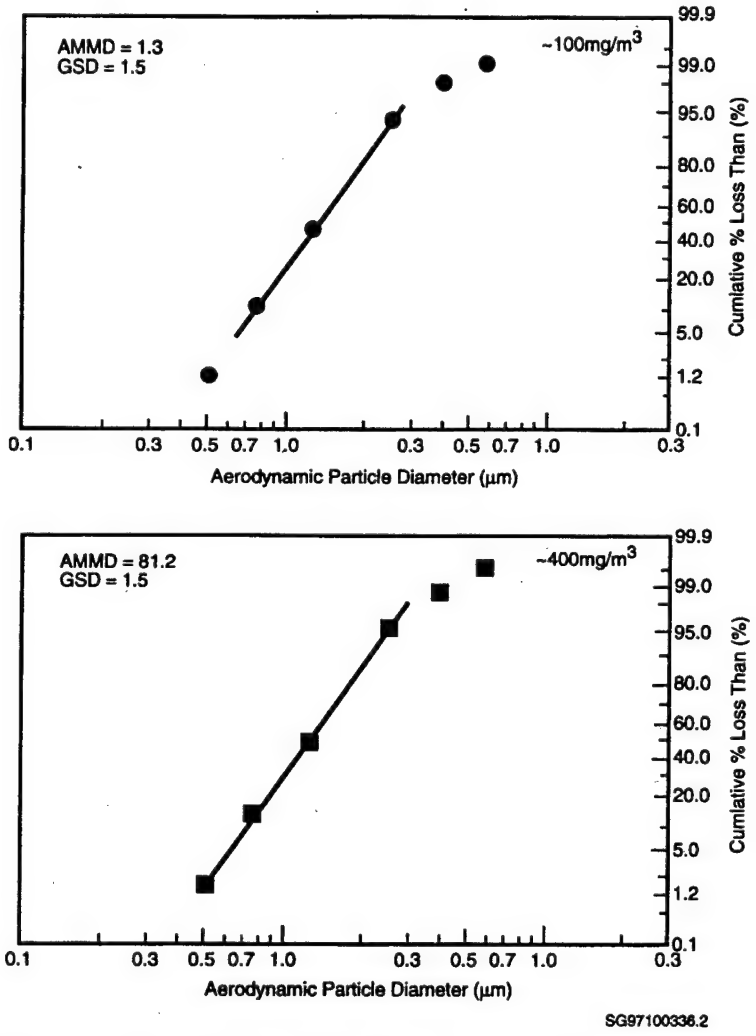

Figure 3. Aerodynamic Mass Median Diameter (AMMD) and Geometric Standard Deviation (GSD) of the 4-hour 100 mg/m^3 (top) and 400 mg/m^3 (bottom) exposure aerosols.

The airborne concentrations of the PAH constituents were estimated for the 400 mg/m^3 fog oil aerosol using the concentration ratios determined from the deposition coupons (Table 2). The sum of the airborne concentrations of all of the low and high weight PAHs was about 0.2 mg/m^3. For more field-typical exposures (100 mg/m^3 fog oil), the PAH total concentration is estimated to be about 0.005

mg/m³. The combined concentration of the more toxic high molecular weight PAHs present during the 400 mg/m³ exposure was less than 0.06 mg/m³ (Table 2). A total PAH concentration of about 0.015 mg/m³ is estimated to be present in a 100 mg/m³ fog oil aerosol.

Table 1. Fog oil composition before and after generation.

	Pre-Generation	Post-Generation Conc (µg/g FO)		
Constituent	Conc (µg/g FO)[a]	2 Hr[b]	2 Hr[b]	3 Hr[c]
Low Molecular Weight PAHs:				
Naphthalene	66.5	<MDL[d]	<MDL	1.8
Acenaphthylene	<MDL	<MDL	1.6	6.0
Acenaphthene	8.9	<MDL	2.6	7.0
Fluorene	32.4	15.2	17.5	31.9
Dibenzothiophene	335	240.0	249.2	302.7
Phenanthrene	165	149.2	152.3	184.5
Anthracene	23.9	24.8	20.9	<MDL
Fluoranthene	14.6	29.5	28.0	24.1
High Molecular Weight PAHs:				
Pyrene	9.1	63.9	57.6	<MDL
Benzo(a)anthracene	<MDL	22.0	<MDL	21.5
Chrysene	52.7	59.2	56.6	55.9
Benzo(b)fluoranthene	<MDL	<MDL	<MDL	<MDL
Benz(k)fluoranthene	<MDL	<MDL	<MDL	<MDL
Benzo(e)pyrene	<MDL	<MDL	<MDL	<MDL
Benzo(a)pyrene	<MDL	<MDL	<MDL	<MDL
Perylene	<MDL	<MDL	<MDL	<MDL
Indeno(123-cd)pyrene	<MDL	<MDL	<MDL	<MDL
Dibenzo(a,h)anthrancene	<MDL	<MDL	<MDL	<MDL
Benzo(g,h,i)perylene	<MDL	<MDL	<MDL	6.5

(a) Airborne concentration at generation was 400 mg/m³. Samples were collected on aluminum foil deposition coupons. No constituents were found on unused foil blanks or on the foil blanks placed in the control chambers for 3 hours during generation.

(b) Detection limit for PAHs is 1.5 µg/g.

(c) Detection limit for PAHs is 0.91 µg/g.

(d) Less than the minimum detection limit.

Table 2. Estimated airborne concentrations of polynuclear aromatic hydrocarbons in fog oil aerosols.

Constituent	Mean Concentration ($\mu g/m^3$)[(a)]
Low Molecular Weight PAHs:	
Naphthalene	0.5
Acenaphthylene	1.1
Acenaphthene	1.3
Fluorene	8.1
Dibenzothiophene	99.2
Phenanthrene	60.9
Anthracene	5.9
Fluoranthene	10.2
High Molecular Weight PAHs:	
Pyrene	15.4
Benzo(a)anthracene	5.6
Chrysene	21.5
Benzo(g,h,i)perylene	1.0

(a) Airborne concentration of individual constituent at generation of the 400 mg/m^3 aerosols estimated from deposition composition (Table 1).

Fog Oil Deposition on Bird Feathers and Estimated Oral Dose

No fog oil was found (< detection limit of 1 μg/g) on the feathers of taxidermic birds exposed for 75 minutes to airborne fog oil concentrations of 113 mg/m^3 or less. Fog oil deposition of 240 to 470 μg fog oil/g of feathers was observed on feathers of birds exposed to 376 mg fog oil/m^3 for 85 minutes (Table 3). These data indicate a fog oil deposition rate of 2.8 μg/g feathers/min to 5.5 μg oil/g feathers/min for birds sitting in aerosols of about 400 mg/m^3.

With deposition rates of fog oil on feathers of 2.8 to 5.5 μg/g/min, oil accumulations of 0.7 mg/g of feathers to 1.3 mg/g of feathers could be expected for birds exposed to 400 mg/m^3 for 4 hours. Typically, birds contaminated in oil spills preen between 20 percent and 50 percent of the oil deposited to feathers within an 8-hour period (Hartung 1963, 1967). The worst case scenario assumes that birds would ingest, from preening, 100 percent of the feather-deposited fog oil. Using Equation 1 (page 21), the dose range for adult male red-cockaded woodpeckers, exposed for 4 hours to fog oil concentrations of 400 mg/m^3, is estimated to be from 52 to 96 mg/kg. These doses are about 10 to 20 times lower than those found to be harmful to birds ingesting unrefined oils (Hartung and Hunt 1966; Chia 1971). Significant pathologies from preening unrefined petroleum oils have

been observed only in waterfowl obtaining doses of at least 1 ml/kg (Hartung and Hunt 1966; Chia 1971). No toxic or histopathological lesions were associated with fog oil exposure in the current study. Therefore, preening does not appear to be a toxicologically significant route of uptake for birds exposed to aerosolized fog oil used in military exercises.

Table 3. Fog oil deposition and PAH concentrations on bird feathers from taxidermic birds exposed to fog oil smoke.

	Aerosol Concentration (mg/m^3)						
				400			%
Constituent	0	50[b]	100	Bird 1	Bird 2	Bird 3	Recovery
Low Molecular Weight PAHs:[a]							
Naphthalene	<MDL[c]	63.2	111	528	523	345	124
Acenaphthylene	<MDL	<MDL	<MDL	52.8	74.0	94.1	95
Acenaphthene	<MDL	<MDL	27.2	36.2	40.4	40.1	94
Fluorene	<MDL	<MDL	68.4	<MDL	<MDL	113	95
Dibenzothiophene	<MDL	54.5	203	193	361	339	NA
Phenanthrene	<MDL	<MDL	131	146	262	240	85
Anthracene	<MDL	<MDL	47.1	60.0	86.9	74.6	91
Fluoranthene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	109
High Molecular Weight PAHs:[a]							
Pyrene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	108
Benzo(a)anthracene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	102
Chrysene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	100
Benzo(b)fluoranthene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	141
Benz(k)fluoranthene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	131
Benzo(e)pyrene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	NA
Benzo(a)pyrene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	113
Perylene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	NA
Indeno(123-cd)pyrene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	92
Dibenzo(a,h)anthrancene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	90
Benzo(g,h,I)perylene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	84
Fog Oil:							
Total on Feathers (µg/g)	<MDL[d]	<MDL	<MDL	240	470	390	NA

(a) Values are in ng/g of feathers, unless otherwise indicated.
(b) Nominal concentration values; actual values are 53 mg/m^3, 113 mg/m^3, and 376 mg/m^3.
(c) Less than the minimum detection limit (20 ng/g).
(d) Less than the minimum detection limit (1 µg/g).

PAH Deposition on Feathers and Estimated Oral Dose

Differences in toxicity observed for various crude oils and other petroleum products in birds appear to be due to the variability in the aromatic composition of the oils (Leighton 1982). The oral doses of the individual and combined PAHs for the blackbirds were estimates because the toxicity of petroleum oil is largely attributed to PAH content (Hoffman 1979; Patton and Dieter 1980; Peakall et al. 1981, 1982). Table 3 lists the concentrations of low molecular weight (≥2 and <4 benzene rings) and high molecular weight (>4 benzene rings) PAHs found on feathers of taxidermic birds exposed to fog oil aerosol.

No detectable levels of any high molecular weight PAHs were found on taxidermic birds exposed to the 400 mg/m^3 smoke. The total concentration of low molecular weight PAHs found on feathers was less than 1.4 μg/g of feathers (Table 3). A linear relationship ($r^2 = 0.85$) was found between the deposition rate (ng of total PAH/g feathers/min) of PAH on feathers and the airborne fog oil concentration (Figure 4). This relationship was used to predict oral uptake of low molecular weight PAHs caused by preening contaminated feathers. For an airborne concentration of 400 mg/m^3, the rate of PAH deposition would be about 14 ng/g feathers/min. Assuming the worst case scenario described in the section on "Fog Oil Deposition on Bird Feathers and Estimated Oral Dose" (see page 28), a male red-cockaded woodpecker could receive an oral dose of low molecular weight PAH of about 0.25 mg/kg after 4 hours of exposure.

In studies with herring gull nestlings, the toxicities of two crude oils were found to be restricted entirely to the aromatic fraction of the oils (Peakall et al. 1981, 1982). Further separation of the aromatic fraction demonstrated the toxicity of the crude oils resided in the fraction containing compounds with four or more benzene rings (the high molecular weight PAHs). No effect on growth, organ weight, or plasma sodium level was observed in gulls receiving the low molecular weight fraction. The concentration of PAHs given to the birds was the amount extractable from 1 g (0.04 oz) of the parent crude oil. Although no concentration for the low molecular weight compounds was reported, the effective dose can be roughly estimated from composition values for crude oils. Crude oil typically consists of 15 to 40 percent aromatic hydrocarbons; about 1 to 10 percent of the oil consists of the high molecular weight PAHs. From these data, the dose of low molecular weight PAHs that did not result in adverse effects in the gulls was, conservatively, at least 10 mg (1 percent of 1 g of crude oil) per bird. The weight-adjusted dose for the 400-g (12.9 oz) gull nestlings would be about 25 mg/kg of body weight. This very conservative no-effect dose is 10 times higher than the dose previously estimated for the current study and indicates that preening a

concentration of fog oil high enough to affect body or organ weights is highly unlikely. A study by Holmes and Cronshaw (1977) showed that daily oral doses of 5 ml of crude oil or No. 2 fuel oil for 90 days did not cause body or organ weight changes in mallards. Again, the PAH concentrations ingested by the ducks without adverse effect far surpasses probable oral uptake from the preening route in birds exposed to fog oil aerosols. This conclusion is substantiated by the lack of adverse effects observed in the red-winged blackbirds after 4 hours of fog oil smoke exposure (see "Response Measures" on page 32).

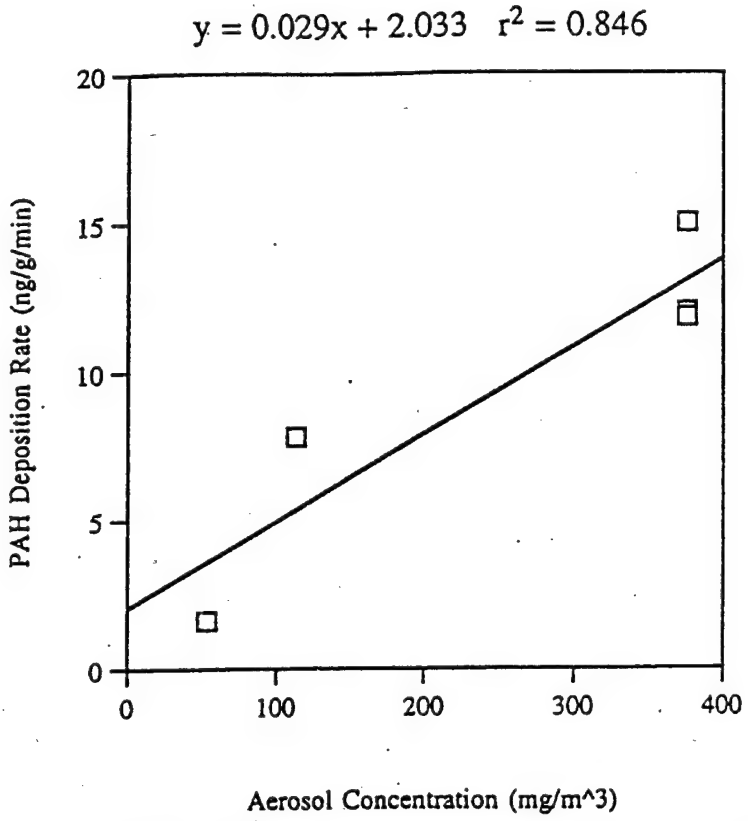

Figure 4. Relationship between PAH content of feathers and exposure concentration.

Estimated Threshold Hypothermic Dose From Feather Deposition

The effects of external oil contamination of feathers include the loss of thermal insulation. The subsequent increase in metabolic rate to maintain body temperature (Hartung 1967) results in depletion of fat and muscular energy resources (Croxall 1977). The lowest reported dermal dose to cause exhaustion of energy reserves and a fall in core body temperature in water birds, is 6 ml/kg body weight (Hartung 1967). The same thermal deficits occur in birds out of water but are somewhat less pronounced, particularly if abundant food sources are available (Hartung 1967).

Given a specific gravity of 0.91 g/ml (and not accounting for physiological scaling between species), the threshold dermal dose for a 44-g (1.4 oz) female red-cockaded woodpecker is about 5.5 g/kg, or 240 mg/bird. For a male red-cockaded woodpecker, the threshold hypothermic dose would be about 310 mg/bird. These threshold doses are reduced, however, when interspecific physiological scaling is considered. Application of a scaling factor of 1.15 (Mineau, Collins, and Baril 1996) between a duck and a red-cockaded woodpecker would result in predicted threshold doses of 150 mg/bird and 190 mg/bird for female and male woodpeckers, respectively. With a deposition rate of 1.3 mg fog oil per gram of feathers, Equation 1 (page 21) estimates show that red-cockaded woodpeckers would receive a dermal dose of about 4.2 mg/bird for females and 5.4 mg/bird for males. These values are far below the predicted dermal hypothermic threshold doses for petroleum oil. Thus, the deposition rate of fog oil aerosols to feathers precludes obtaining an acute dose that would result in a drop in core body temperature under typical training scenarios. This conclusion appears to be supported by the present study.

Body temperature and food consumption were not directly measured during the study. Compared to controls, no behavioral indications were found of body heat loss (fluffed feathers and heat seeking), increased feeding activity, body weight loss, or diminished carcass condition (wasted fat and muscle tissues) in the red-winged blackbirds exposed to fog oil.

Response Measures

None of the treated birds showed adverse response to fog oil aerosols. Gross and histopathological lesions were found but were equally distributed among control, as well as test birds. The lesions were unrelated to fog oil exposure and were associated with disease exposure typical of wild bird populations.

Mortality, Clinical Signs of Toxicity, and Behavioral Abnormalities

Birds exposed for up to 4 hours to about 400 mg/m^3 of SGF-2 did not die or exhibit clinical signs of toxicity at any time during the exposure or post-exposure period. During exposure, the birds sat, stood, or perched quietly. They did not exhibit any signs of stress (fluffed feathers, gaping, lowered postural stances). After exposure, no behavioral abnormalities were observed, including those associated with hypothermia, general stress, or oil-specific postural changes (see page 31) for oiled birds or for birds that were fed diets containing petroleum products.

Although the inhalation toxicity of petroleum oils has not been previously characterized for birds, an aerosol form of naphthalene (a constituent of petroleum products) has been found to be highly toxic to psittacine species (Harrison and Harrison 1986). The airborne concentration of naphthalene was estimated (see Table 2, page 28) to be very low (about 0.5 $\mu g/m^3$) in the generated obscurant aerosols. As discussed previously (see pages 30 and 31), the amount of aromatic constituents deposited on exposed birds was also insufficient to cause adverse effects via oral or dermal uptake.

Gross Pathology

All birds were in good body condition at necropsy. No muscular atrophy or depleted body fat was observed in the control or treated birds. The plumage of the birds exposed to fog oil smoke was in good condition and did not differ in sheen or shape from that of control birds. No evidence of diarrhea or vent staining was found and no exudates from the eyes or nares were seen. Air sacs were clear in all birds. Occurrences of gross lesions were few and widely distributed among the treatment groups (Table 4).

Table 4. Incidence of gross lesions in red-winged blackbirds exposed to SGF-2.

	Lesion			
Treatment (mg/m^3)	Inflamed Ileum[a]	Parasitic Worm	Enlarged Spleen[b]	Lesion on Bill
2-Hour Exposure				
0	1/16[c]	--	--	--
50	--	--	--	--
100	--	--	2/16	2/16
400	1/16	2/16	--	1/16
4-Hour Exposure				
0	--	--	--	--
50	--	--	--	--
100	--	1/16	--	--
400	--	2/16	--	--

(a) Inflammation in both cases appeared mild.

(b) Spleens appeared to be moderately enlarged.

(c) Number of birds with lesion/number of birds in test group.

A wide spectrum of pathology has been associated with acute toxicity of refined hydrocarbons. The most commonly reported visceral lesion is hemorrhagic gastroenteritis (Crocker, Cronshaw, and Holmes 1974; Langenberg and Dein 1982).

Most of the other gross lesions associated with petroleum exposure are the same as those reported for birds debilitated by stress. They include urate nephropathy and visceral gout, enlarged hemorrhagic adrenal glands, and focal myocardial degeneration (Crocker, Cronshaw, and Holmes 1974; Miller et al. 1978; Miller Peakall, and Kinter 1978; Pattee and Franson 1982; Szaro et al. 1978; Szaro, Hensler, and Heinz 1981). Only two blackbirds appeared to have developed lesions that could be associated with hydrocarbon exposure or debilitating stress (intestinal inflammation), and one of those was a control bird (Table 4). The remaining incidence of gross pathology was attributable to parasite infestation. Three birds were found with a single encrusted papule in the lore region of the head between the eye and bill. These birds were in the 100 mg/m^3 and 400 mg/m^3 exposure groups of the 2-hour exposure test. No birds in the 4-hour exposure test developed the papulae. Thus, it appears that exposure to high concentrations of SGF-2 obscurant smoke for up to 4 hours does not result in any sublethal gross pathology in red-winged blackbirds.

Histopathology

Table 5 lists the histopathological lesions found in red-winged blackbirds 5 days and 28 days post-exposure to fog oil aerosol. Lesions were limited to a low incidence of a variety of liver abnormalities, proliferation of lymphoid tissue in the lung and other organs, and parasitic infection.

Table 5. Occurrence of histopathological lesions in red-winged blackbirds exposed to SGF-2.

Treatment	Lymphoid Nodules			Parasites		
(mg/m^3)	Lung	Proventriculus	Liver	Liver	Worms	Coccidia
2-Hour Exposure						
0	6/8[a]	3/8	2/8	0/8	1/8	8/8
50	5/8	0/8	2/8	2/8[b]	1/8	8/8
100	5/8	1/8	1/8	0/8	2/8	7/8
400	8/8	3/8	3/8	1/8[c]	0/8	6/8
4-Hour Exposure						
0	3/8	3/8	4/8	1/8[d]	1/8	6/8
50	2/8	7/8	2/8	1/8[b]	0/8	8/8
100	3/8	2/8	1/8	0/8	1/8[e]	8/8
400	2/8	2/8	1/8	1/8[c]	0/8	8/8

(a) Number of birds with lesion/number of birds sampled
(b) Vacuolated
(c) Congested
(d) Multinecrotic foci
(e) Associated inflammatory response (macrophages, lymphoid cells, heterophils) also present

A wide variety of histopathological changes in organ morphology have been documented in oiled birds (Hartung and Hunt 1966; Beer 1968; Pattee and Franson 1982; Snyder, Fox, and Soare 1973; Szaro et al. 1978; Szaor, Hensler, and Heinz 1981). The lesions reported varied in type and severity among the studies and among the birds within the studies. However, the lesions common among oil-treated birds appear to be intestinal necrosis, pneumonia, and renal tubule nephrosis. No intestinal or kidney damage was observed among the birds in the present study. No evidence of intestinal damage was observed at the tissue level in infected birds, although intestinal coccidiosis was present in most of the blackbirds (Table 5). Using an arbitrary scale based on the number of coccidia present, the majority of the birds appeared to have only a mild protozoal infection limited to enteric tissues. Other studies with wild birds have reported subclinical, yet severe, coccidia lesions in the intestine with protozoal invasion of the liver, heart, and other organs (Carpenter et al. 1979; Fleming, Sileo, and Franson 1982). Extensive coccidial invasions of the intestine and several other organs were reported in sandhill cranes experimentally dosed with 2 or 10 mg/kg crude oil (Fleming, Sileo, and Franson 1982). As in the current study, the infection was observed in both control and oil-dosed birds equally and followed a subclinical course independent of the oil treatments. Other parasites (nematodes and tapeworms) were scattered among test groups of blackbirds and also appeared to be independent of fog oil exposure.

Lung lesions have been observed in birds that have aspirated crude oil and include tracheal epithelium with a metaplastic appearance, cells that lack cilia, and a granulomatous inflammatory response in lung tissue characterized by macrophages laden with phagocytized oil (Butler et al. 1988). Although these lesions were not found in the blackbirds, proliferation of lymphoid nodules associated with the bronchial epithelium of the submucosa was observed in 53 percent of the birds sampled (Table 5). A change in lymphoepithelial tissue is documented in waterfowl that were fed diets containing petroleum; however, the response was an involution rather than the proliferation of the lymphoepithelial tissues seen in the blackbirds (Butler et al. 1988). Also, the incidence of lymph node proliferation was equally distributed among control and treated birds and was independent of fog oil exposure. In addition, such nodules are commonly found in domestic and wild birds and probably represent antigenic exposure, particularly in response to such infectious agents as Mycoplasma spp. (Yoder 1984). A low incidence of lymphoid proliferation in the proventriculus and liver was also widely distributed between the oil-treated and control blackbirds (Table 5).

Blackbirds in this study presented a low incidence of liver abnormalities (Table 5). Although mild degenerative changes in the liver were observed in a number of wild or experimentally oiled birds (Hartung and Hunt 1966; Beer 1968; Snyder, Fox, and Soare 1973; Szaro et al. 1978; Szaro, Hensler, and Heinz 1981; Pattee and Franson 1982), the occurrence of the liver lesions, as with all the observed lesions in the blackbirds, appears unrelated to fog oil exposure.

Body Weight

Mean body weights of male and female birds during the acclimation and test periods are presented in Tables 6 through 9. Throughout the study, body weights for males and females were typical for red-winged blackbirds found in the region. At nearby Columbia National Wildlife Refuge, the body weight of 2500 adult male blackbirds ranged from 60 to 88 g (1.9 to 2.8 oz) with a seasonal mean body mass of 74.1 ±3.8 g (Beletsky 1996). Females ranged in weight between 36 and 65 g (1.2 and 2.1 oz) with a seasonal mean of 47.4 ±3.4 g (Beletsky 1996). In the present study, the mean body weight was 73 ±5.6 g for males and 49 ±7.8 g for females.

Throughout the study period differences in body weight between control and treated birds were not observed in either the 2-hour or the 4-hour exposures The males in all test groups, including the controls, exhibited a transient increase in body weight between 2 weeks pre-test and Day 0 of the study. The weight gain was not observed in females that were weighed randomly among the males. The period of weight increase in the males coincided with the end of the breeding season and a reduction in male territorial displays and other reproductive behavior. A similar weight gain has been observed in post-breeding males in field studies (Beletsky 1996), presumably as a result of reapportioning time from breeding and territorial defense activities to foraging. However, body weights in the captive birds appeared to return to pre-test weights by Day 5 and may be a further example of the seasonal and individual variation in body mass. Large daily changes (4 percent) in body mass have been reported in male red-winged blackbirds and birds of similar size (Beletsky and Orians 1987; Owen 1954, Kacelnik 1979).

Table 6. Mean body weights (in grams) of adult male red-winged blackbirds exposed to fog oil aerosols for 2 hours.

		Treatment Group (mg/m^3)				
Time Interval	n	0	50	100	400	Anova P Value
2 Weeks Pre-Test	8	70	70[a]	68	71	0.57
Day 0 of the Study	8	77	82	74	78	0.05 [b]
Day 5 Post-Exposure	8	68	73	73	65	0.12
Day 28 Post-Exposure	4	68	71	67	66	0.51

(a) n=10; At necropsy, two birds, phenotypically identified as females, were instead juvenile males.

(b) Mean body weight of each treatment group was not significantly different (P≥0.05) from control mean; Two-Tailed Dunnett's Test.

Table 7. Mean body weights (in grams) of adult female red-winged blackbirds exposed to fog oil aerosols for 2 hours.

		Treatment Group (mg/m^3)				
Time Interval	n	0	50	100	400	Anova P Value
2 Weeks Pre-Test	8	52	50[a]	48	55	0.64
Day 0 of the Study	8	54	46	45	56	0.35
Day 5 Post-Exposure	8	58	48	45	52	0.87
Day 28 Post-Exposure	4	55	50	47	51	0.47

(a) n=6; At necropsy, two birds, phenotypically identified as females, were instead juvenile males.

Table 8. Mean body weights (in grams) of adult male red-winged blackbirds exposed to fog oil aerosols for 4 hours.

		Treatment Group (mg/m^3)				
Time Interval	n	0	50	100	400	Anova P Value
2 Weeks Pre-Test	8	71	69	71	71	0.76
Day 0 of the Study	8	75	75	80	74	0.31
Day 5 Post-Exposure	8	72	73	79	69	0.43
Day 28 Post-Exposure	4	69	70	68	68	0.90

Table 9. Mean body weights (in grams) of adult female red-winged blackbirds exposed to fog oil aerosols for 4 hours.

		Treatment Group (mg/m^3)				
Time Interval	n	0	50	100	400	Anova P Value
2 Weeks Pre-Test	8	46	48	53	47	0.15
Day 0 of the Study	8	49	48	52	52	0.17
Day 5 Post-Exposure	8	46	42	47	49	0.14
Day 28 Post-Exposure	4	43	44	48	49	0.70

Reduced growth in nestlings (Miller et al. 1978; Miller, Pekall, and Kinter 1978; Peakall et al. 1982) has been reported for wild birds ingesting petroleum. Miller et al. (1978) attributed the decreased growth rate to reduced nutrient transport in the intestine. However, other authors have failed to observe changes in growth (Szaro 1977; Szaro and Albers 1977; Gorman and Simms 1978; Eastin and Rattner 1982). Decreased body mass in adults has not been induced by treatment with petroleum (Rattner 1981; Pattee and Franson 1982). The conflicting reports may be a result of the use of different crude oils and refined products, different dosing regimes, and the varying ages of the birds (Clark 1984). As discussed in the paragraphs on estimating the threshold hypothermic dose (see page 31), oral uptake of fog oil and its aromatic constituents was small and less than the concentrations shown to cause weight loss or other adverse effects. Inhalation and dermal uptake during exposure to fog oil smoke, combined with the preening dose, were apparently below levels that diminish body mass and condition.

Liver Weight

Liver weights of the control and treated blackbirds were not significantly different (Table 10) and were similar to those reported for normal birds on a per body weight basis (Miller et al. 1978; Miller, Peakall, and Kinter 1978). Although hypertrophy of hepatic tissue with a single oral dose of petroleum has been demonstrated (Miller et al. 1978; Miller, Peakall, and Kinter 1978; Peakall et al. 1982), the high molecular weight PAHs appear to be the causative constituents of the oils tested (Peakall et al. 1982) and are not detectable in aerosols of fog oil (see "PAH Deposition on Feathers and Estimated Oral Dose" on page 30).

Table 10. Liver weights as percentages of adult red-winged blackbirds exposed to fog oil aerosols for 4 hours.

		Treatment Group (mg/m^3)				
Day Post-Exposure	n	0	50	100	400	Anova P Value[(a)]
Males						
Day 5	4	2.8	2.3	2.8	2.5	0.11
Day 28	4	2.9	2.5	2.5	2.4	0.28
Females						
Day 5	4	3.0	2.7	1.9	3.1	0.74
Day 28	4	3.1	2.9	2.6	3.1	0.11

(a) Anova was applied to the arcsine transformation values for the tissue weight proportions.

Respiratory Function

The mean respiratory frequency and minute ventilation of male red-winged blackbirds prior to fog oil exposure was 93 ±7 breaths/min and 0.045 ±0.005 L/min, respectively. The range of minute volumes for the male birds was from 0.042 to 0.055 L/min. During the 2-hour exposure test, the respiratory rate and minute ventilation of the birds exposed to 400 mg/m^3 of fog oil were 91 ±10 breaths/min and 0.042 ±0.07 L/min; n=3), respectively. These values are similar to those observed prior to exposure and to the control males (95 ±7 breaths/min and 0.046 ±0.007 L/min; n=4). The mean pre-exposure respiratory rate for female blackbirds in the 2-hour test was 97 ±8 breaths/min (n = 6) with a range of 89 breaths/min to 102 breaths/min. The mean minute ventilation for the females was 0.037 ±0.013 L/min (0.024 L/min to 0.05 L/min). Because of difficulty in restraining the birds in the holding tubes during exposure (see page 23), insufficient data were collected for the other test groups and we were unable to obtain respiratory values for birds during the 4-hour exposure.

To monitor the respiratory function response of the blackbirds during the 4-hour exposure, respiratory rates of the free-ranging birds in the chambers were obtained by visual observation (number of chest movements/15 seconds multiplied by 4). No differences in respiratory frequency were seen between oil-exposed birds and controls in the 4-hour test (Table 11). Measurement of respiratory frequency by direct observation of unrestrained birds is considered to be more accurate than that obtained from birds restrained in a plethysmograph when conditions of thermal neutrality (air temperatures of 22 to 26° C [2.2 to 4.5 °F]) are met (Calder 1968). However, the accuracy of the blackbird data is suspect because of the difficulty in observing the birds through the smoke in the darkened chamber. Even with illumination, counting such small chest wall movements is difficult. A flashlight was used to illuminate the birds through a small flap in the wall covering of the exposure chamber, but direct illumination of the birds resulted in a gaping behavior. It was difficult to indirectly illuminate sufficient birds and reliably count chest wall contractions. Direct measurements of post-exposure respiratory function for the blackbirds are not available because frequency data could not be consistently obtained under comparable conditions (thermal neutrality and social density) in the outdoor aviary.

Assuming the pre-exposure minute ventilation is an adequate estimation of the ventilatory rate of the exposed birds, a bird the size of a male red-winged blackbird would have inhaled about 52 mg/kg of fog oil during the 4-hour exposure test. The inhalation dose of a 49-g (1.6 oz) bird (a female red-winged blackbird) would be about 68 mg/kg.

Although respiratory function data are limited, lack of clinical signs of respiratory stress during exposure and post-exposure periods and the absence of oil-induced lesions at gross necropsy and at histopathological examination of respiratory tissues indicate that respiratory damage by SGF-2 aerosols in birds is minimal.

Table 11. Mean respiratory frequency of adult red-winged blackbirds exposed to fog oil aerosols for 4 hours.

		Treatment Group (mg/m^3)			
Time[a]	n	0	50	100	400
15-30	3	99 ±6[b]	94 ±7	97 ±6	94 ±6
210-245	3	101 ±4	96 ±6	97 ±5	93 ±8

(a) Minutes from start of the 4-hour exposure that the respiratory rates were taken.

(b) Values are in breaths/min ±SD.

4 Conclusions

Generation of fog oil results in changes in chemical composition of the oil. Primarily, the concentration of acutely toxic naphthalene is reduced by more than 97%; the number and concentration of high molecular weight PAHs is increased. However, the airborne concentrations of the high molecular weight PAHs and the PAH deposition to feathers is very low to undetectable. Estimated oral doses of oil and PAHs from preening were much lower than levels reported to cause hypothermia, gross pathologies, tissue damage, or death.

Compared to the controls, no mortality, clinical signs of toxicity, abnormal behavior, weight loss, or gross or histopathological lesions related to fog oil exposure were observed in any of the test birds. Therefore, it appears adult red-winged blackbirds (a surrogate species for birds with similar respiratory ventilation, feather mass, and dermal surface areas) can sustain fog oil exposures of about 400 mg/m^3 for 4 hours with no detectable adverse effects.

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Appendix: Exposure Profiles for 2-hour and 4-hour Whole-body Exposures of Red-winged Blackbirds to Fog Oil (SGF-2)

All concentrations were calculated from curve shown in Figure 2 and real-time IDS readings.

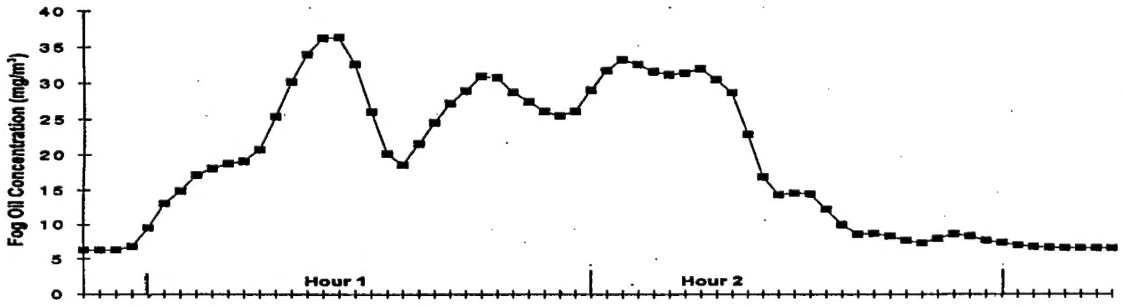

Figure A-1. Exposure profile for fog oil during the nominally 50 mg/m^3 2-hour exposure test.

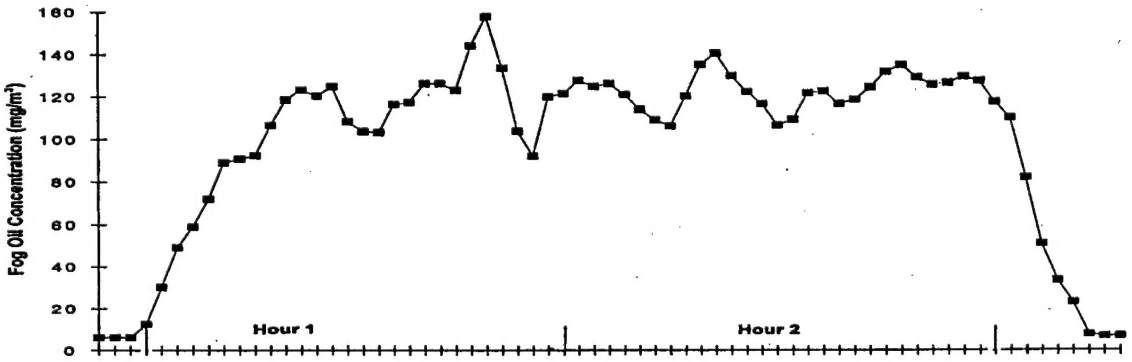

Figure A-2. Exposure profile for fog oil during the nominally 100 mg/m^3 2-hour exposure test.

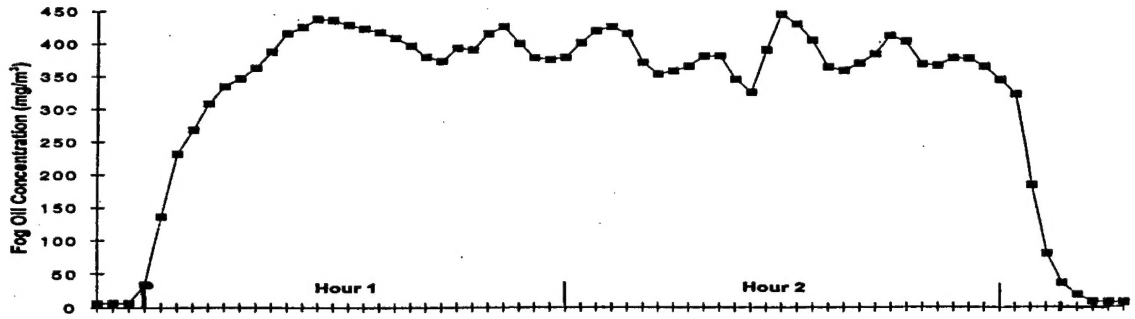

Figure A-3. Exposure profile for fog oil during the nominally 400 mg/m^3 2-hour exposure test.

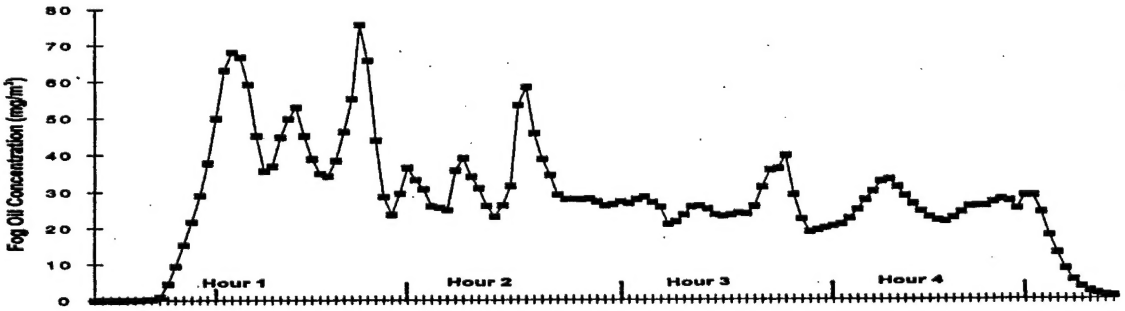

Figure A-4. Exposure profile for fog oil during the nominally 50 mg/m^3 4-hour exposure test.

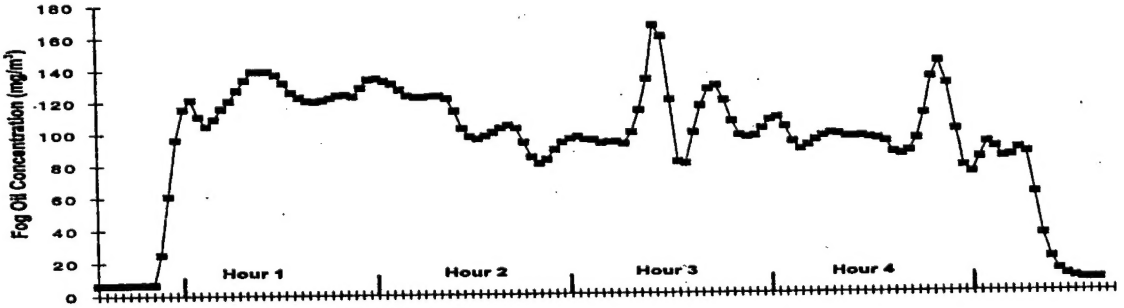

Figure A-5. Exposure profile for fog oil during the nominally 100 mg/m^3 4-hour exposure test.

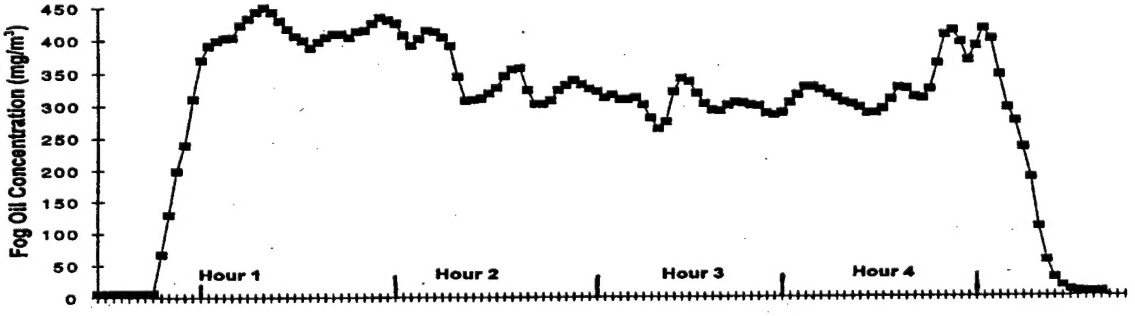

Figure A-6. Exposure profile for fog oil during the nominally 400 mg/m^3 4-hour exposure test.

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19
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Acute Inhalation Toxicity of Fog Oil Smoke in the Red-winged Blackbird, a Size-specific Inhalation Surrogate for the Red-cockaded Woodpecker

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14. ABSTRACT

The red-cockaded woodpecker (*Picoides borealis*) is an endangered species often found on military lands where troop readiness training is conducted. An important component of this training is the generation of obscurant materiel to conduct maneuvers under obscurant cover. Fog oil (SGF-2) is the obscurant most commonly deployed for these purposes. To help assess and manage the impact of fog oil to sensitive wildlife species, such as the red-cockaded woodpecker, we evaluated the acute toxicity and health risk of environmental releases of fog oil to avian wildlife. The red-winged blackbird (*Agelaius phoeniceus*) was used as a surrogate species for the red-cockaded woodpecker.

Wild red-winged blackbirds were exposed to varying airborne concentrations of fog oil. The post-exposure response of the blackbirds was monitored for 28 days. Compared to control birds, no mortality, body weight loss, clinical signs of toxicity, or behavioral abnormalities were observed in any of the fog oil-treated birds. The distribution and severity of gross and histopathological lesions were independent of the fog oil exposures and were typical of those found in wild bird populations. Although respiratory function data were limited, no major respiratory impairment or abnormalities were observed during the exposure or the post-exposure period.

15. SUBJECT TERMS
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